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ORIGINAL ARTICLE

Breeding biology aspects of spotted flapshell Turtle, *Lissemys punctata* (Lacepede 1788), in Bangladesh

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KEYWORDS

Breeding biology;
Spotted flapshell turtle;
Clutch size;
Incubation;
Hatching success

Abstract This study was conducted to determine the breeding season, gonadal development, egg laying period, clutch size and other biological aspects of spotted flapshell turtle, *Lissemys punctata*, of Bangladesh between January 1997 and December 2001. The egg laying period of *L. punctata*, was found between August and March. The nesting sites were elevated fallow lands in secluded areas. The female turtle laid all the mature eggs at a time for each clutch at night. A gravid female turtle laid three clutches of eggs in each year and the mean clutch size was 13.0 ± 1.9 eggs and mean weight of each egg was 10.3 ± 1.3 g. The eggs are spherical in shape and whitish in color. The mean incubation period was 173 ± 34 days (range 119–225 days). The incubation period of first clutch was the longest than the second and third clutches. Hatching success was found 41%. Maximum hatching was observed in June. The present investigation was made to explore the possibility to raise turtle farming in captive condition. The findings would, hopefully, help to rear the species and to assess the commercial potentiality of turtle farming in captive condition, that is, in the eco-climatic condition of Bangladesh.

1. Introduction

Freshwater turtles are consumed by a group of people as a source of protein for its delicacy (Rao, 1987). As turtle meat and turtle products are very delicious, its demand is increasing progressively. Further, turtles are an ancillary exportable commodity, Bangladesh exported 3164.24 metric tons of freshwater turtles in the fiscal years 1980–2002 (Export Promotion Bureau of Bangladesh, 2001–2002). Unsustainable exploitation is of a great threat of extinction in near future (Sarker and Hossain 1997). Turtle population is declining due to over exploitation and habitat destruction (Rao, 1986) and rare turtle species are also collected for food (Zhao and Adler, 1993). The declination rate is a serious indication as some species have been rapidly declining in the past decade, may face extinction in the wild (Sandra and Daniela, 2000). Once turtles species were very prevalent

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in different ecoclimatic conditions of Bangladesh; however, in the last few decades, due to over exploitation that is, viz., random hunting, illegal catching and destruction of habitat and breeding ground destruction, etc., the population has been decreasing very rapidly (Hossain, 2000). Turtle species are indispensable for the sustenance of different ecosystems, in as much as, it works as a scavenger in different ecosystem, aquatic and terrestrial habitat in particular; thereby keep the water quality pollution free (Sarker and Hossain, 1995).

Breeding in hatchery condition could be an appropriate solution to replenish the natural population of turtle (Hossain et al., 2005). By far, a little information is available on breeding biology of *Lissemys puctata* in country. Some works have, however, been done on distribution and biology of *L. punctata* by Khan (1982), Fugler (1984), Barua and Islam (1986), Hossain and Sarker (1995a) and Rashid and Swingland (1997). Therefore, it is felt that artificial breeding in the hatchery could be an appropriate solution to sustain the population as well as to cater the market demands.

2. Materials and methods

A study on breeding biology, that is, breeding season, breeding ground, nest building, egg laying, clutch size, incubation period, hatching, predation, growth of hatchlings of the spotted flapshell turtle was done in nature at Matlab Upazila under Chandpur district; Sonargaon and Siddirganj Upazilas under Narayngonj district; Gopalganj and Madaripur districts and in captivity at the Zoological garden of the Department of Zoology, Dhaka University; Gopinathpur Upazila under Manikganj district and in north Kamalapur, Dhaka.

2.1. Observations on breeding activities in captivity

A total of thirty-nine specimens of gravid spotted flapshell turtle was collected from natural habitat of Matlab and Sonargaon and reared in enclosures of Zoological garden of Dhaka University as well as 2 mini turtle hatcheries at Arua Union, Manikganj district, and 18/1, Kabi Jasimuddin Road, north Kamalapur, Dhaka. The nesting activity was closely observed at day and night. An artificial culture conditions were created in the Zoological garden, a cemented tank ($4 \times 4 \times 4.5$ m) with 3.5 m width within the boundary of the tank which was used by the turtles as roaming, feeding and breeding ground. Two mini ponds of 1683 m, each ($8 \times 7 \times 3$ m) of which was used to rear and to observe the breeding activities of the turtles. The corners of the enclosure, in the zoological garden, were slightly elevated and middle was slightly sloped inserted with 6 earthen jars (100 cm^2) with ideal habitat, the device facilitated the turtle species to get out and get in easily to the jars. Some plants (arum) were grown up inside the enclosure that provided shade for the turtle. There were some breeding grounds made in the corner of the enclosure with different type of soil to estimate the nesting preferences.

2.2. Observations on breeding activities in nature

Nesting sites and nests and other breeding activities were identified through close observation in nature. Information regarding breeding aspects was also collected from local people and turtle catchers through a set of interview schedule. Information was also gathered by accompanying the turtle collectors in the study areas. Clutch size was calculated by enumerating the eggs laid in nests in nature.

2.3. Breeding season

Breeding season was determined by monitoring the breeding activities in nature as well as in captivity. Turtle catchers, consumers and local people were interviewed through questionnaires in the study areas. Gravid *L. punctata* were reared at the Zoological garden, and hatcheries of Arua Union, Manikgonj and Jasimuddin Road for observing the egg laying time and other breeding activities in captivity. The maturity of eggs was calculated by pressing finger through the folds of hind limbs.

2.4. Gonadal development, clutch size, egg laying period and measurement of eggs

To observe gonadal development, eggs maturity, eggs number, length-girth-diameter of eggs, etc., at least 05 specimens were dissected monthly throughout the study period. A total of 258 specimens of *L. punctata* were dissected in the field and laboratory. Besides, 287 dead turtles were collected from local markets (Matlab Fish Market, Baidar Bazar, Patkhel Bazar, Bholtoli Hat, Satpar Hat, Betgram Hat, Hatiara Hat, Baniarchar Bazar, Jaliarpar Bazar of Gopalganj; Kadambari Bazar, Zitka Bazar-Manikganj) and export centers (Mirpur 10, Uttara 14 No. Section, Battoli-Narayanganj) and dissected them to know the gonadal development, egg maturity, clutch size and conditions of oviducts. The presence or absence of mature and immature eggs in both oviducts helped to determine clutch size. The first egg laying period or first clutch was defined by the presence of mature and immature eggs; the second egg laying period was ascertained when more mature eggs and few immature eggs were found in both oviducts, and third egg laying period was determined when no immature eggs other than mature eggs in the oviducts. Electric balance and vernier calipers were used to measure weight, length, girth and dimension of eggs. The relationships between clutch size and body weight were analyzed and the regression lines were drawn with the regression equation $Y = a + bx$. [Where Y = clutch size (dependent variable), b = regression coefficient, a = constant value.]

2.5. Incubation period

The incubation period was calculated from the day of keeping the eggs underneath the soil. Loam, sandy loam, clay, silt clay soil were used in the steel trays with antifungal treatment and breeding grounds to study the incubation and hatching performances. Temperature and moisture were recorded by using soil thermometer and spot tester regularly. Hatching success was calculated by enumerating the number of hatchlings in relation to the eggs laid per clutch.

2.6. Statistical analysis

Data were processed under computer software MS Excel, using relevant statistical methods like correlation and regression, significance was tested at $p < 0.05$ and $p < 0.05$ level.

3. Results and discussion

3.1. Egg laying

In the present study, the egg laying period of *L. punctata* was found between mid August and mid March. Fugler (1984) was apparently the first worker to report that the turtle laid eggs in the month of December, which is a good agreement with the present study. However, the observation is not consistent with the findings of some other workers. It was found that one gravid female *L. punctata* laid three clutches of eggs in each year. First clutch of eggs laid between mid August and mid October, second clutch between December and mid January and third clutch was between February and March. Both the oviducts were developed and contained mature and immature eggs in the beginning of August. No mature egg was found in the oviducts between the months of April and July during the study period. Whitaker and Andrews (1997), and also Rashid and Swingland (1997) mentioned that *L. punctata* shown the breeding behaviour round the year. Das (1995) collected 12 clutches of eggs from Crocodile Bank Trust; the eggs were laid in August to December. Goin and Goin (1971) reported that nesting has been observed in July to September early in the monsoon. Breeding period may vary due to variations of environmental influences.

3.2. Nesting sites

It was observed that nesting sites of *L. punctata* were in the elevated fallow land, grass lands along the village roads, bank of ponds and canals, edge of embankments, backyard garden, elevated portion of the graveyard, bamboo groves, shade of rain tree and palm tree, narrow demarcating paddy fields and corner of school yard. Usually the sites were selected in secluded places with sparse vegetation. The nesting sites of *L. punctata* were in loam soil (76%) and less in sandy (24%) soil. Nests were 12–850 m (mean 399.5 ± 287 m) away at the height 5.5–9.6 m above the surface of waterbodies ($n = 12$), where water does not stand for long time. Das (1995) mentioned that the nest of *L. punctata* usually being shallow pit about 8–10 cm deep situated under the cover of thorny bushes. Hossain and Sarker (1995a) reported that *L. punctata* made nests in the ground with the help of claws and head, and leave the nesting place by labelling the soil with plastron. According to Vijaya (1981) nesting was made in the mound surround by thick growth of bushes and grass, receiving filtered sunlight. The present study showed that most of the nests were made in fallow lands in secluded areas with sparse vegetation. But in captivity, the female turtles used the corner, slightly elevated, of the enclosure, rim of the mini ponds and cemented tank at the zoological garden for nesting. It was noted that only the gravid females selected the nesting sites.

3.3. Vegetation around the nests

Some vegetations e.g. *Clerodendron infortuntum* (Bhat), *Solanum torvum* (Titbegun), *Mentha viridis* (Pudina), *Centella asiatica* (Thankuni patha), *Cynodon dactylon* (Durba), *Adhatoda vasica* (Bhashak), *Foeniculum vulgare* (Mouri), *Vernonia cineria* (Sheal mutra), Ghora catla, Pahari lata, Dhansha, *Cajanus cajan* (Arahar dal) were recorded in and around the nesting sites.

3.4. Nest building

In nature, the female turtles made their nests ($n = 11$) in loam moist soil. Nests were made with the help of sharp claws of limbs and the protrusive neck. The turtle concealed the eggs in the nest, and labeled and pressed the nest soil by the plastron.

In captivity, the nests were made between 18 and 23 min (mean 20.5 ± 1.8 min; $n = 8$) at Zoological garden, Dhaka University. A turtle made a nest within 20 min and laid 16 eggs then these eggs were hidden with the help of hind limbs in a nursery ground of Aura Union, Manikganj.

3.5. Nest depth and diameter

In nature, the depth of the nest varied from 8.8 cm to 10.6 cm (mean 9.8 ± 0.6 cm) and the diameter varied between 9.9 cm and 12.0 cm (mean 11.0 ± 0.6 cm) (Table 1). The greater the clutch size the higher the diameter and the lower the depth of the nest. The clutch size of the turtle varied from 10 to 16 eggs (mean 13 ± 2.0) (Table 1). The relationship between the nest diameter and nest depth were negatively correlated ($r = -0.947$) and the relation was insignificant ($t = 2.228$ at $p < 0.05$). The nest diameter and clutch size was significantly correlated ($r = 0.830$, $t = 5.961$ and $p < 0.01$) and the nest depth and clutch size was negatively correlated but significant ($r = -.864$, $t = 2.375$) at 1% level. From the regression line it was evident that the nest diameter and clutch size ($Y = 0.3375$, $x + 6.6792$, $R^2 = 0.8847$) and the nest depth and clutch size ($Y = 0 - .2313$, $x + 12.84$, $R^2 = 0.7996$) were correlated (Fig. 1). Das (1995) mentioned that the nest dimension of *L. punctata* was 8.9×8.5 cm and 20.0×21.0 cm (length × breadth) and 14.8 and 9.8 cm depth. Vijaya (1981) mentioned that the depth of first exposed eggs was 8 cm, the nest chamber 8 cm in diameter and the depth of nest was 12 cm.

3.6. Egg laying sequence and period

In nature, the female *L. punctata* laid all mature eggs at a time in the self-made nest. Two female turtles were found to lay their eggs along side the village road, and bank of a pond in the village Bhaznakhal and Kaladhi, Chandpur district at 20:50 h and 23:40 h, respectively. A female was found making nest beside the village path at 20:20 h in the village Dingabanga, Matlab; another female was found nesting at the edge of embankment of the Meghna Dhonaghoda Irrigation

Table 1 Measurements of nests of spotted flapshell turtle, *Lissemys punctata*.

	Nest diameter (cm)	Nest depth (cm)	Clutch size (No.)
	12.0	8.9	16
	11.8	8.8	15
	11.6	9.4	14
	11.3	9.7	13
	10.8	10.1	14
	10.7	10.4	13
	10.5	10.2	11
	10.7	10.6	10
	9.9	10.5	11
Mean ± SD	11.0 ± 0.6	9.8 ± 0.6	13 ± 2.0

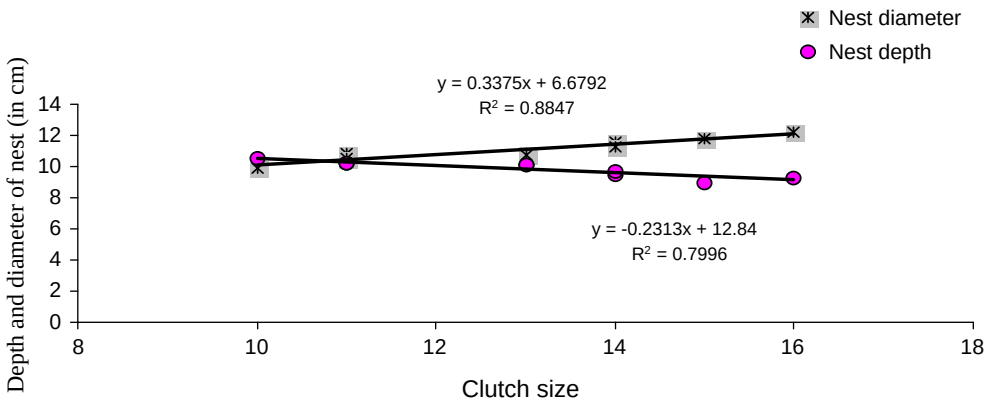

Figure 1 Correlation of clutch size with depth and diameter of the nest of *Lissemys punctata*.

Project, Matlab at 21:15 h. It was noted that the eggs laying time to be at night from 20:00 h to 24:00 h. Chen (1976) reported that *L. punctata* made holes in the sand and laid eggs but he did not mention the laying time.

In captivity, one female turtle laid 16 eggs at the corner of enclosure at 21:30 h on 19th August, another laid 14 eggs in an earthen jar at 23:30 h on 24th August. Furthermore, another two females laid 11 eggs in the nursery bed at 23:35 h on 21st December and 13 eggs in a cell in the zoological garden at 21:25 h on 3rd January, respectively. Three gravid female turtles were released in the zoological garden, one laid 16 eggs in the water of earthen jar on 18th August, and rest two turtles made nests in the corner of the enclosure and laid 15 to 13 eggs on 23rd and 27th August as first clutch, respectively. Of the three turtle, one laid 12 eggs on 18th December in the corner of the mini pond another two laid 13 to 10 eggs at the corner of the cemented tank on 25th and 29th December as second clutch, respectively. These three females laid 12 eggs, 11 eggs and 10 eggs on 17th February, 3rd and 7th March as third clutch. All the eggs were laid at night.

During the present study it was noted that the female turtles started laying first clutch in August, second clutch in December and the third clutch ended in March in a single breeding season. It was ascertained on the basis of dissection, there was no shelled egg in the oviducts of the turtles just after laying first clutch except the maturing and immature ones ($n = 13$). But after laying the third clutch of eggs no mature and immature eggs were found in the oviducts ($n = 15$). Alderton (1997) stated that if the females were to lay more than one clutch of eggs, as one set matures and it's laid, so another comes closer to completing the period in the ovary. Once expelled from one ovary, the ova may actually transfer to the other oviduct. It could possible serve to balance the number of ova present within the oviduct. The produced ova are not followed immediate egg laying, it can take several weeks, possibly as long as two months complete the final stage of their development. It is a good agreement to the present findings. On 4th April a *L. punctata* contained shelled eggs inside the oviducts; the egg retention in the oviduct has been attributed to unfavorable weather conditions or lack of proper conditions for egg laying.

3.7. Clutch size

In nature, the clutch size varied from 10 to 18 eggs (mean 13.0 ± 2.1). The egg laying sequence was higher in the first clutch than the second and third ones. On the basis of dissection of 63 gravid female turtles in different places at different times it was found that the clutch size varied between 10 and 17 eggs (mean 14.03 ± 0.9) (Table 2). The weight of dissected turtles varied from 880 to 2230 g (mean 1565 ± 244.5 g). Auffenberg (1981) mentioned that female usually produced two clutches of eggs per season but some times three clutches; body size and egg size was negatively correlated. This findings supported by Duda and Gupta (1978). Clutch of early season having large number and more elongated eggs than later ones. The present findings support the statements but confirmed that eggs were spherical in shape. The clutch size did not increase always with female size. The relationship between the body weight and clutch size was negatively correlated ($r = -.519$, $t = 1.62$ and $p < 0.05$).

Table 2 Clutch size of *Lissemys punctata* determined by dissection α.

Sample size (turtle)	Mean weight of dissected turtles (g) ± SD	Total eggs found	Range (eggs)	Mean clutch size ± SD	Relationship between body weight and clutch size (*r*)	'*t*-statistic' (calculated)	'*t*-statistic' (tabulated)
14	1561.8 ± 308.6	192	10–17	13.7 ± 2.0	0.411	1.56 ns	2.16
07	1820.0 ± 239.4	103	13–16	14.7 ± 1.1	0–.081	2.51[a]	2.44
08	1347.5 ± 188.8	121	12–17	15.1 ± 1.6	0.242	0.61 ns	2.36
04	2015.0 ± 42.8	61	13–17	15.3 ± 1.7	0.872	2.85[a]	2.77
05	1674.0 ± 193.1	67	11–15	13.4 ± 1.5	0.616	1.36 ns	3.18
09	1481.0 ± 515.1	117	10–16	13.0 ± 2.1	0.586	1.91 ns	2.77
11	1397.3 ± 312.6	155	10–17	14.1 ± 2.1	0.182	0.56 ns	2.22
05	1224.0 ± 264.3	65	10–15	13.0 ± 1.9	0.637	1.31 ns	3.16

[a] Significant at 5% level, $\alpha = n = 63$ and ns = insignificant.

The clutch size of *L. punctata* was 5–12 eggs (Whitaker and Andrews, 1997), 2–8 eggs (Das, 1995), 10–12 eggs laid (Smith, 1931), 2–6 eggs within few weeks (Deraniyagala, 1953), 2–14 eggs and a female lays several clutch in each year (Barbour and Ernst, 1989). But, Fugler (1984) mentioned the clutch size was 10–12 eggs but he did not mention the number of clutch in a year. Kuchling (1988) mentioned that the soft-shelled freshwater turtles *Erynnochelys madagascariansis* laid eggs into three different clutches. Das (1995) collected 12 eggs from Madras Crocodile Bank, India, he also mentioned that the turtle laid 6–14 eggs at a time. Rashid and Swingland (1997) stated that female lays three or several clutches in each year and the clutch size was 2–14 eggs (mean 8.1 ± 0.03). Clutch size was increased with female size, the egg size remains almost same and there was a decreasing trend in eggs size with female size (Yadava and Prashad, 1982). Present study confirms that there was a decreasing trend of clutch size with female size.

3.8. Shape and colour of eggs

The shape of eggs of *L. punctata* was spherical like tennis ball. The shell was delicate, fine and its texture was slightly glossy that supported by Chen (1976), Das (1995) and Vijaya (1981). The fresh eggs were usually whitish in colour, sometimes brownish. The colour became pale at the later part of incubation when embryos became mature in the eggs.

3.9. Measurement of eggs

The weight of individual eggs varied from 5.5 g to 12.6 g (mean 10.3 ± 1.3 g; $n = 100$). The weight varied in each clutch of eggs of the same female turtle (Table 3). The diameter of eggs varied from 21.1 mm to 28.4 mm (mean 26.0 ± 1.4 mm). Egg dimension was also varied in each clutch. It was found that there was decreasing trend in egg size with female size and the dimension of the eggs was also increased when weight of eggs increased. The relationship between the eggs weight and dimension was positively correlated and statistically significant ($r = 0.880$, $n = 100$, $t = 18.53$ and $p < 0.05$). The relationship between egg weight and diameter maintained linearity. The linear regression of dimension of eggs with weight was ($Y = 1.0347$, $x + 15.152$ and $R^2 = 0.9742$) correlated and 97.4% weight variation can be explained (Y) by the values of eggs dimension (X) (Fig. 2), where R^2 = is the accuracy coefficient.

Table 3 Measurement of eggs of spotted flapshell turtle, *Lissemys punctata* ($n = 100$).

Clutch size	Weight (g)		Diameter (mm)	
	Range	Mean ± SD	Range	Mean ± SD
11	5.5–10.8	9.32 ± 1.5	21.1–26.9	25.3 ± 1.5
12	8.1–11.1	9.76 ± 0.9	24.4–27.7	26.0 ± 0.9
13	7.1–11.3	9.63 ± 1.2	21.9–27.3	25.2 ± 2.0
14	8.5–12.5	10.30 ± 1.1	24.7–27.4	26.2 ± 1.0
15	8.3–12.1	10.13 ± 1.2	22.1–27.4	25.7 ± 1.5
10	8.2–11.7	10.21 ± 0.9	23.0–26.8	25.5 ± 1.2
12	10.6–12.6	11.62 ± 0.6	25.8–27.5	26.9 ± 0.6
13	10.5–12.6	11.36 ± 0.7	24.9–28.4	27.0 ± 1.0

Das (1995) mentioned that the eggs of *L. punctata* measuring 25–33 mm and weight 12.2–13.8 g. Chen (1976) stated that diameter of eggs was 1.5–3.0 mm, Vijaya (1981) reported as 24–33 mm, Deraniyagala (1953) mentioned as 30–33 mm and weight 17–19.5 g, Barbour and Ernst (1989) reported as 24–33 mm in diameter. The egg size was 24.1 mm but there was a decreasing trend in the egg size with body size (Yadava and Prashad, 1982).

3.10. Incubation

The incubation period varied between 131 and 224 days (mean 173 ± 33.7 days). The incubation period was longer for the first clutch of eggs than the second and third clutches. The incubation period ranged for the first clutch from 206 to 224 days (mean 218 ± 6.2), second clutch 148–169 days (mean 160.7 ± 6.7) and third clutch ranged from 131 to 141 days (mean 136.6 ± 3.9) (Table 4). Factors were noted involved in maintaining the incubation period (e.g., temperature, humidity, nest depth, soil quality, shade) were considered. The incubation period was longer when the temperature and humidity were lower in the nest (Fig. 3).

3.11. Temperature of nests

The mean temperature in the nest varied between 26 °C and 30 °C (mean 28.4 ± 1.0 °C). The temperature varied due to rainfall and drought during the incubation period. Optimum temperature had been shorten the incubation period. First clutch of eggs hatched in longer period with minimum temperature. The mean temperature for first clutch varied 26 °C to 27.5 °C (mean 27.3 ± 0.4 °C); second clutch 28 °C to 29.5 °C (mean 28.8 ± 0.7 °C) and third clutch from 29 °C to 30 °C (mean 29.4 ± 0.4 °C) (Table 4). The incubation period was

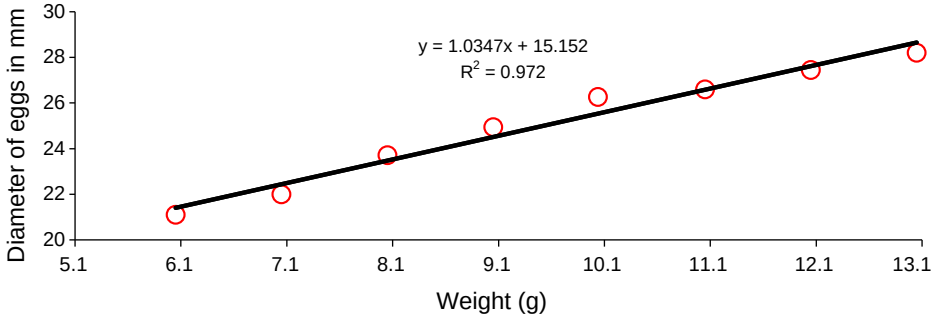

Figure 2 Relationship between weight and dimension of eggs of *Lissemys punctata*.

Table 4 Incubation period of eggs of *Lissemys punctata* as affected by environmental conditions and the nest depth.

Clutch	Clutch size (eggs)	Nest temperature range (°C)	Mean temperature (°C)	Nest humidity range (%)	Mean humidity (%)	Nest depth (cm)	Incubation period (days)	Hatchling (in number)
First	16	24.0–32.0	26.0	65–91	87	6.8	223	3
	15	25.0–31.5	27.0	66–90	84	6.4	221	6
	14	24.5–30.0	27.5	67–90	88	7.4	224	3
	13	24.5–30.0	27.5	64–92	83	5.9	220	3
	14	25.0–32.5	27.5	65–86	78	7.3	214	5
	14	25.0–30.5	27.5	65–88	89	7.0	206	4
Second	13	26.5–31.0	29.5	64–91	90	7.5	162	5
	10	25.0–30.0	29.0	68–87	79	7.1	157	6
	14	26.0–30.5	29.5	70–88	86	7.6	158	7
	16	25.5–30.0	27.5	69–87	88	5.7	168	7
	12	25.0–31.0	28.0	68–90	87	6.7	163	7
	16	24.5–30.5	28.5	71–91	88	7.2	169	9
	12	26.0–31.0	29.5	70–91	89	5.9	148	4
Third	15	26.5–32.5	30.0	70–92	89	6.7	141	7
	11	25.0–31.0	29.0	72–91	91	6.7	139	5
	11	25.0–32.0	29.5	79–94	90	7.2	133	6
	10	26.0–30.5	29.0	79–93	90	7.1	139	6
	13	25.5–31.5	29.5	78–94	90	6.9	131	5

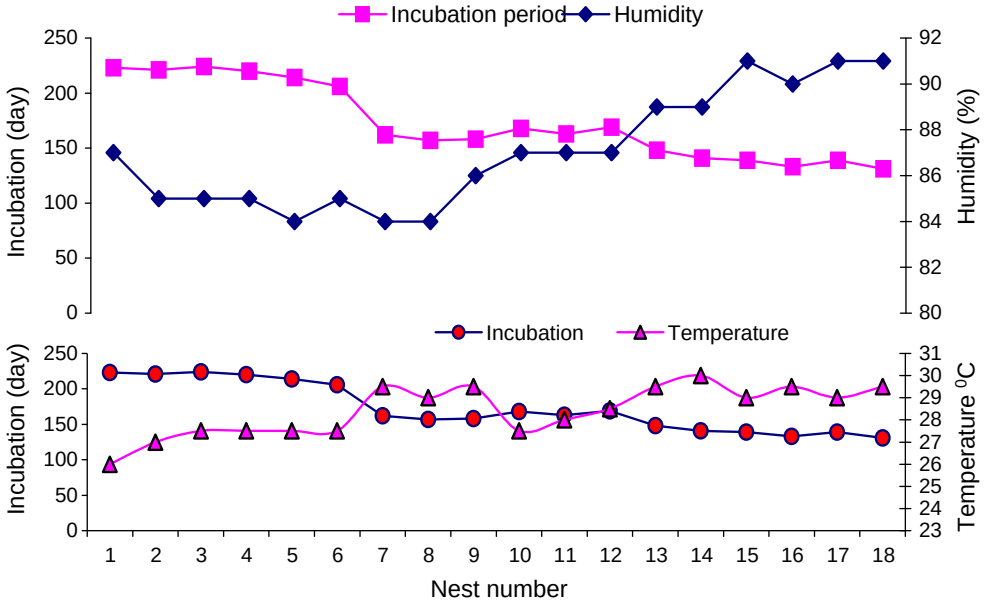

Figure 3 Incubation period of eggs of *L. punctata* a affected by environmental conditions in the nest.

50 days with 30 °C in an average (Chen, 1976); about 9 months at 32.2 °C in the nest and 32.6 °C in air (Vijaya, 1981); 60–230 days for in north India but the duration may vary 60–260 days depending on before or after winter nesting (Whitaker and Andrews, 1997). The period required for incubation differed from one place to another due to environmental difference.

3.12. Humidity of nests

The mean humidity in the nest varied from 78% to 91% (mean 86.4 ± 3.9%). The third clutch of eggs hatched with short time then the first and second clutch of eggs. The mean humidity for first clutch ranged from 78% to 89% (mean 84.8 ± 3.7%), second clutch 79–90% (mean 85.3 ± 3.8%) and third clutch 89–91% (mean 90 ± 0.6%). It is confirmed that optimum humidity reduced the length of incubation period (Table 4).

The relationship between incubation period and temperature was negatively correlated significantly ($r = -0.875$) and between incubation and humidity was also negatively correlated ($r = -0.490$) significantly. The relation between incubation and nest depth was negatively correlated ($r = -0.075$). The incubation and clutch size was correlated ($r = 0.516$) but significantly. The relation between temperature and hatching success was non significantly correlated ($r = 0.28$). The incubation and hatching success was negatively correlated

($r = -0.388$) and significant (Table 5). The incubation period was short when temperature was high at the same time humidity was minimum (Fig. 3).

3.13. Hatching time

Hatching time was from April to July and the highest was in May (34.7%) (Table 6). Hatchlings did not emerge out before rains that coincide with period of high water levels and abundance. The sound of thunder appears to act a cue (Das, 1995). Hatching occurs in April to May (Rashid and Swingland, 1997). The hatchlings of *L. punctata* emerge just prior to arrival of the monsoon i.e. from mid May to mid June (Rao and Singh, 1987).

3.14. Soil quality of nest

The present experimental study showed that hatching success depends on soil texture e.g. loam, sandy and clay characteristics. Maximum numbers of hatchlings were found from loam soil with partial shade. The percentage of hatching was 62% in loam soil but most of eggs were dried up and formed vacuum in the eggs shell in the sandy and moist less soil.

Table 5 The correlation coefficient (r) and testing with environmental parameters in incubation period of eggs of *L. punctata*.

Parameters	Value of r	't'-statistic' calculated value	Statistical inference
Incubation: temperature (°C)	0–.875	2.35	*
Incubation: humidity (%)	0–.490	3.16	**
Incubation: nest depth	0–.075	3.93	*
Incubation: clutch size	0.516	2.42	*
Temperature: hatching success	0.237	0.98	ns
Humidity: hatching success	0–.388	3.38	*

ns = non-significant.
* $p < 0.05$.
** $p < 0.01$.

3.15. Sunlight and moisture of the nests

Some eggs were kept under the soil in hatching trays where sunshine penetrated directly. The eggs were not hatched out but the yolk and albumen became dry and the colour of egg shell became pale. Some eggs were spoiled due to heavy moisture in the nesting ground for a long time. Diapause was occurred for twelve eggs when a nest was covered with hard layer of mud, the hatchlings were dig out from the breeding ground after 13 days of the same base of eggs of this turtle.

3.16. Hatching success and hatching in successive months

A total of 98 hatchlings were emerged out from 239 eggs, so hatching success was 41%. Two clutches of eggs were absolutely spoiled. There was no clutch of eggs produced 100% nestlings but 59% of eggs did not produce any nestling (Table 6). Of the hatchlings 24 (24.5%) were hatched out in April, 11 (11.2%) in May, 34 (34.7%) in June and 29 (29.6%) in July (Table 6). The hatching success was 10.1% in April, 4.6% in May, 14.2% in June and 12.1% in July in relation to eggs laid in successive months (Table 6).

3.17. Predation

Monitor lizards, mongooses, crows, kites, red ants were noted as the major predators for eggs and hatchlings. One hatchling was found in the stomach of Grey land monitor at Hatiya Island (Hossain et al., 1995). During the study period, 21 nestlings and 88 eggs were preyed by these predators. This finding supports the findings of Bhupathy and Vijayan (1989). Sivasubramanian and Bhupathy (1990) mentioned that Adjutant stork as predator of spotted flapshell turtle. Alderton (1997) mentioned the snakes are the main predators, which devours the eggs and young of turtles but they did not mention the species name.

Table 6 Percentage of hatching of eggs of *Lissemys punctata* in successive months.

Hatching time	No. of eggs laid	Young hatched	Relation to young hatched (%)	Hatching success to eggs laid (%)	Hatching success in relation to eggs laid (%)
April	16	03		18.8	
	15	06		40.0	
	14	03		21.4	
	13	03	24.5	23.1	10.1
	14	05		35.7	
	14	04		28.6	
May	10	05		50.0	
	13	06	11.2	46.2	4.6
June	14	07		50.0	
	16	07		43.8	
	12	07	34.7	48.3	14.2
	16	09		56.3	
	12	04		33.3	
July	15	07		46.7	
	11	05		45.5	
	11	06	29.6	54.5	12.2
	10	06		60.0	
	13	05		38.5	

3.18. Mortality and rearing

In rearing stage in captivity the mortality rate was higher in first year than second year. There was no mortality in third year. The highest growth of hatchlings occurred in 3rd year and attained between 290 g and 448 g in captive condition. The post-hatching mortality recorded to be higher (Passmore and Ronald, 1993). Alderton (1997) mentioned that captive breeding turtles often tend to breed at an earlier age than their counterparts as they usually grow at a faster rate. Although the availability of food has been suggested as a determining factor.

3.19. Breeding success

The total number of eggs laid, the young hatched and rearing of hatchlings was calculated. Two hundred thirtynine eggs of 18 clutches were kept in the ground and 98 young were emerged out. So the hatching success was 41% in relation to the eggs laid. Of the hatchlings, 21 were lost by predation, 3 cannibalized and 27 died due to handling, fungal infection and other incidents. So the hatchery success was 19.67% in relation to hatchlings emerged out and escaped from predation and other incidences.

In view of increasing demand, the artificial breeding of *L. punctata* in captivity should be practised to meet the demand in the market and to reduce the pressure on the natural population. Bangladesh has a vast land having open and closed freshwater sources where this species could thrive. The present study revealed that the obtained biological information could be applied in successful breeding of the species under controlled conditions.

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